

**CHITINASE PRODUCTION BY CHITINOLYTIC BACTERIA FROM
CATFISH**

(Clarias gariepinus)

BY

ONIBOKUN, ADEOLA ELIZABETH

13PCQ00506

**DEPARTMENT OF BIOLOGICAL SCIENCES
SCHOOL OF NATURAL AND APPLIED SCIENCES
COVENANT UNIVERSITY**

MAY, 2015

CHITINASE PRODUCTION BY CHITINOLYTIC BACTERIA FROM CATFISH

(*Clarias gariepinus*)

BY

ONIBOKUN, ADEOLA ELIZABETH

13PCQ00506

**A DISSERTATION SUBMITTED TO THE MICROBIOLOGY PROGRAMME OF
THE DEPARTMENT OF BIOLOGICAL SCIENCES SCHOOL OF POSTGRADUATE
STUDIES**

COVENANT UNIVERSITY, OTA,

OGUN STATE, NIGERIA

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
AWARD OF MASTER'S DEGREE IN MICROBIOLOGY**

MAY, 2015.

DEDICATION

I dedicate this project work to God Almighty, The Giver of life, my Sustainer, Provider of all resources and most of all the supplier of my strength, and wisdom without whom this work would not become a reality.

DECLARATION

I, ONIBOKUN ELIZABETH ADEOLA, hereby declare that this project work was carried out by me in the department of biological sciences, school of natural and applied sciences, college of science and technology under the supervision of Dr A. A. Ajayi.

This project work has not been submitted anywhere else for a degree award. The ideas are majorly products of a research conducted by me. All ideas from other authors have been duly acknowledged.

ONIBOKUN ELIZABETH ADEOLA

Researcher

Signature and Date

CERTIFICATION

We certify that this project work was carried out by ONIBOKUN ELIZABETH ADEOLA, 13PCQ00506 in the Microbiology programme of the Department of Biological Sciences, School of Natural and Applied Sciences, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria.

Signature Date

Dr. A. A Ajayi

(Project supervisor)

Signature Date

Dr. E. E. J. Iweala

(HOD, Biological Sciences)

Signature Date

Prof. Charles Ogbologo

(Dean, School of Postgraduate studies)

Signature Date

Prof. Bola Adeniyi

(External Examiner)

ACKNOWLEDGEMENT

I return all the glory to God for the success of this work. My gratitude also goes to my supervisor, Dr A. A Ajayi for her constructive criticisms, motherly advice and dedication throughout the period of this work. I will also use this medium to appreciate all my lecturers, Prof. Egwuari, Prof. Nandita , Dr Oranusi, Dr Owolabi, Dr lawal, Dr Olaseinde, Dr Ayepola, Dr Omonhinnin for all their support and encouragement. I also appreciate the efforts of all technologists in the Department of Biological sciences. May God reward you all.

Finally, I appreciate the efforts of my parents for their financial contributions, my siblings, friends, colleagues and Miss Adedeji, Odia Trust for their numerous contributions to the success of this work.

It is my prayer that the favour of God will not depart from your lives. Thank you and God bless you all in Jesus name. Amen.

TABLE OF CONTENT

TITLE	PAGE
TITLE PAGE.....	I
DEDICATION.....	III
DECLARATION.....	IV
CERTIFICATION.....	V
ACKNOWLEDGEMENT.....	VI
TABLE OF CONTENT.....	VII
LIST OF PLATES.....	XII
LIST OF FIGURES.....	XIII
LIST OF TABLES.....	XV
ABSTRACT.....	XVI
CHAPTER ONE	
1.0 INTRODUCTION.....	1
1.1 STATEMENT OF THE PROBLEM.....	1
1.2 JUSTIFICATION OF THE RESEARCH.....	2
1.3 OBJECTIVES OF THE RESEARCH WORK.....	3
CHAPTER TWO	
2.0 CHARACTERISTICS OF ENZYMES.....	4
2.1 MICROBIAL ENZYMES.....	4

2.2 CHITINASE.....	5
2.2.1 CLASSIFICATION OF CHITINASE.....	6
2.2.2 PRODUCTION OF CHITINASE.....	6
2.3 APPLICATION OF CHITINASE IN INDUSTRY.....	7
2.3.1 CHITINASES AS BIOPESTICIDES.....	7
2.3.2 PRODUCTION OF SINGLE CELL PROTEIN.....	8
2.3.3 CHITINASE IN WASTE MANAGEMENT.....	8
2.4 CHITIN.....	9
2.4.1 CHITOSAN.....	10
2.5 THE AFRICAN CATFISH (<i>Clarias gariepinus</i>).....	11
2.5.1 SCIENTIFIC CLASSIFICATION.....	11
2.5.2 DESCRIPTION OF THE AFRICAN CATFISH.....	11
2.5.3 CULTIVATION OF AFRICAN CATFISH.....	13
2.5.4 FEEDING PATTERN OF AFRICAN CATFISH.....	13
2.5.5 MICROBIAL PROFILE OF AFRICAN CATFISH.....	14
2.5.6 ECONOMIC IMPORTANCE OF AFRICAN CATFISH.....	15
CHAPTER THREE	
3.0 MATERIALS.....	16
3.1 METHODS.....	17
3.1.1 COLLECTION OF SAMPLES.....	17
3.1.2 PREPARATION OF CATFISH SAMPLES.....	17

3.1.3 ISOLATION OF BACTERIA POPULATION FROM THE GUT OF CATFISH.....	17
3.1.4 ISOLATION OF BACTERIA POPULATION FROM THE SKIN OF CATFISH.....	17
3.1.5 SELECTION OF PURE CULTURES.....	18
3.1.6 IDENTIFICATION OF BACTERIAL ISOLATES	18
3.1.6.0 GRAM REACTION/MICROSCOPY.....	18
3.1.6.1 MOTILITY TEST/ HYDROGEN SULPHIDE PRODUCTION.....	18
3.1.6.2 SUGAR FERMENTATION TEST.....	18
3.1.6.3 STARCH HYDROLYSIS.....	19
3.1.6.4 UTILIZATION OF UREASE.....	19
3.1.6.5 CATALASE TEST.....	19
3.1.6.6 OXIDASE TEST.....	19
3.1.6.7 METHYL RED VOGUES PROSKAUER TEST (MRVP).....	19
3.1.6.8 UTILIZATION OF CITRATE.....	20
3.1.6.9 INDOLE PRODUCTION TEST.....	20
3.1.7 ISOLATION OF DNA FROM CHITINOLYTIC BACTERIA.....	20
3.1.8 PCR AMPLIFICATION.....	20
3.2.1 PREPARATION OF COLLOIDAL CHITIN.....	21

3.2.2 PREPARATION OF COLLOIDAL-CHITIN AGAR MEDIUM.....	22
3.2.3 CHITINASE PRODUCTION.....	22
3.2.4 COMPOSITION OF BASAL MEDIUM.....	23
3.2.5 ENZYME ASSAY.....	23
3.2.6 PARTIAL PURIFICATION OF ENZYMES.....	24
3.2.7 AMMONIUM SULPHATE PRECIPITATION.....	24
3.3.1 EFFECT OF TEMPERATURE.....	24
3.3.2 EFFECT OF pH.....	25
3.3.3. EFFECT OF SUBSTRATE CONCENTRATIONS.....	25
3.3.4 EFFECT OF TIME OF HEATING.....	25
3.3.5 KINETIC DETERMINATION.....	25
3.3.6 PRIMARY STRUCTURE DETERMINATION.....	26
 CHAPTER FOUR	
4.1 IDENTIFICATION OF BACTERIAL ISOLATES.....	27
4.2 GROWTH OF CHITINOLYTIC BACTERIA.....	27
4.3 CHITINASE PRODUCTION.....	27
4.4 PROPERTIES OF CHITINASE.....	41
4.4.1 EFFECT OF TEMPERATURE.....	41
4.4.2 EFFECT OF pH.....	41
4.4.3 EFFECT OF SUBSTRATE CONCENTRATIONS.....	41
4.4.4 EFFECT OF TIME OF HEATING.....	50

4.5 KINETIC DETERMINATION.....	50
4.6 CHITINASE STRUCTURE DETERMINATION.....	50
4.7 STRUCTURE DETAILS.....	50
4.8 PCR AMPLICATION.....	50
CHAPTER FIVE	
DISCUSSION.....	59
CONCLUSION.....	62
RECOMMENDATION.....	62
REFERNCES.....	62
APPENDIX.....	76

LIST OF PLATES

PLATE	TITLE	PAGE
1	Gel electrophoresis showing RAPD PCR using S30 primer	56

LIST OF FIGURES

FIG	TITLE	PAGE
1	The African catfish (<i>Clarias gariepinus</i>)	12
2	Concrete fish tanks in fish farming village	13
3	Bacteria isolates showing zones of hydrolysis on colloidal chitin agar medium	38
4	Effect of temperature on chitinase enzyme from bacteria specie (Isolate code 17)	42
5	Effect of temperature on chitinase enzyme from bacteria specie (Isolate code 36)	43
6	Effect of pH on chitinase enzyme from bacteria specie (Isolate code 17)	44
7	Effect of pH on chitinase enzyme from bacteria (Isolate code 36)	45
8	Effect of substrate concentration on chitinase enzyme from bacteria specie (Isolate code 17)	46
9	Effect of substrate concentration on chitinase enzyme from bacteria specie (Isolate code 36)	47
10	Lineweaver – Burk plot for hydrolysis of chitin by partially purified chitinase from bacteria specie (Isolate code 17)	48
11	Lineweaver – Burk plot for hydrolysis of chitin by partially purified chitinase from bacteria specie (Isolate code 36)	49
12	Effect of time of heating (70°C) on chitinase enzyme from bacteria	50

	specie (Isolate code 17)	
13	Effect of time of heating (70°C) on chitinase enzyme from bacteria specie (Isolate code 36)	51
14	Effect of time of heating (90°C) on chitinase enzyme from bacteria specie (Isolate code 17)	54
15	Effect of time of heating (90°C) on chitinase enzyme from bacteria specie (Isolate code 36)	55
16	Catalytic domain of chitinase from <i>Bacillus circulans</i> isolated from soil	56
17	Ramachandran plots of the chitinase produced by <i>Bacillus circulans</i> showing the amino acid residues in favorable positions as red and yellow regions and none in unfavorable positions as white regions.	57

LIST OF TABLES

TABLE	TITLE	PAGE
1	Identification of bacterial isolates	29
2	Gram stain reaction	31
3	Sugar fermentation test	32
4	Identification of bacterial isolates with biochemical test	34
5	Diameter of zones of hydrolysis of chitinolytic bacteria	36
6	Partial purification chitinase produced by bacteria specie (Isolate code 17)	39
7	Partial purification chitinase produced by bacteria specie (Isolate code 36)	40

ABSTRACT

Chitinases are hydrolytic enzymes that breakdown the glycosidic bonds in chitin. Chitin is a component of the cell walls of fungi and exoskeletal elements of some animals (including worms and arthropods), therefore chitinases are generally found in organisms that either needs to reshape their own chitin or dissolve and digest the chitin of fungi or animals. The importance of chitinase in industries cannot be overemphasized as it has been applied in agriculture; as a biopesticide for control of plant fungi infections, in medicine; as indicators of fungi infection, and in waste management; for biodegradation of fish waste. The African catfish (*Clarias gariepinus*) which plays host to these chitinolytic bacteria is very readily available and easy to cultivate thus providing a cheap means for the production of chitinase in commercial quantity. Bacteria populations Isolated from catfish were screened on colloidal-chitin agar medium. Chitinase production was determined by zones of hydrolysis produced after 96h of incubation at 37°C. The activity of Chitinase was determined by measuring the amount of reducing sugar released using Dinitrosilylic method. Partial purification of chitinase was carried out by Ammonium Sulphate precipitation. The optimum conditions for the chitinase activity were determined using a number of parameters such as temperature, pH, effect of substrate concentration and the time of heating over 30min. Optimum conditions were therefore ascertained at a temperature of 50°C and a substrate concentration of 0.15g for chitinase produced by bacteria spp (isolate code 17 and 36) while pH 5.5 was obtained for isolate code 36 and pH 6.0 for isolate code 17. The Michaelis – Mentens constant (Km) which is also known as the dissociation constant is the substrate concentration at half maximum velocity. Calculated from the Lineweaver-Burk plot, the apparent Km values for the hydrolysis of chitin by chitinolytic bacterial isolate code 36 and isolate code 17 were approximately 0.09mg/ml and

0.007mg/ml respectively. Isolation of DNA and PCR amplification was carried out on the chitinolytic bacteria used for the study and one of the bacteria was identified as a member of the genus *Bacillus*. This study established that species of *Bacillus* inhabiting the gut and skin of the African catfish can be used to produce chitinase in appreciable quantity. The study has diversified the use of catfish for enzyme production rather than for consumption only. The catfish gut which constitutes a large portion of the fish waste will also serve as a cheap source for chitinase production.